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The Product of *glnL* Is Not Essential for Regulation of Bacterial Nitrogen Assimilation

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The *glnL* product is not necessary for the control of expression of *glnA* and other nitrogen-regulated genes, but it presumably communicates redundant information on the availability of ammonia from an ammonia-sensitive system consisting of the products of *glnB* and *glnD* to the regulatory products of *glnF* and *glnG*.

In *Escherichia coli* and related enteric organisms, the expression of *glnA*, the structural gene for glutamine synthetase, and of genes coding for enzymes capable of providing the cell with ammonia, such as histidase, is regulated according to the quality and abundance of the nitrogen source; this regulation is complex and involves the products of at least five genes: *glnB*, *glnD*, *glnF* (*ntrA*), *glnG* (*ntrC*), and *glnL* (*ntrB*) (5).

Genes *glnA*, *glnL*, and *glnG* are part of the complex *glnALG* operon, which has promoters at the beginning of *glnA* and *glnL* (2–4, 6). Our experiments deal with the regulation of glutamine synthetase and histidase biosynthesis in cells lacking the *glnL* product (Table 1). We have already found that the effects of mutations in *glnB* and *glnD* are not manifested in cells whose *glnL* genes are deleted (2, 5).

The regulation of glutamine synthetase formation in these cells does not differ qualitatively from that observed in wild-type cells; quantitatively, however, ammonia supplied in the medium is somewhat less effective in lowering the level of glutamine synthetase (2) (Table 2, experiments 1 and 2). The lack of the *glnL* product does not prevent the strong repression of glutamine synthetase which results from the inactivation of the *glnF* gene (Table 2, experiments 3 and 4). Thus, this repression which has previously been shown to require the product of the *glnG* gene does not appear to require the product of the *glnL* gene. Conversely, the activation of glutamine synthetase formation requires the product of the *glnF* gene, even in absence of the product of the *glnL* gene. The fact that a strain which carries no *glnL* gene, YMC21 (λ *gln*104), grows well on glucose with arginine or proline as a sole source of nitrogen indicates that the *glnL* product is not required for the activation of the synthesis of ammonia-providing enzymes. Our ability to measure histidase and to quantitate this effect indicated by the growth experiment was prevented by the inability to obtain simple λ lysogens in strains carrying *hut* genes, presumably because of a defect in the λ attachment site in such strains.

It has been shown that insertions in *glnL* completely prevent the expression of *glnG* (4). Apparently, no functional promoter for *glnG* is located downstream from *glnL*. Nevertheless, we found that plasmid pgln31, which carries a 2,000-base-pair region of DNA containing the *glnG* gene and only a portion of the *glnL* gene, could complement a strain with an insertion in *glnG* for normal regulation of glutamine synthetase formation (Table 3, experiments 2 and 3). Since pgln31 lacks the promoters associated with *glnL* and *glnA* (2), *glnG* expression from this plasmid might reflect low-level transcription initiating in pBR322 sequences and proceeding into *glnG*. The level of *glnG* product determined by pgln31 presumably does not vary greatly in response to the nitrogen source of the medium. Although we have not established the mechanism by which pgln31 expresses *glnG*, in subsequent experiments, we employed pgln31 as the source of *glnG* product whose level is relatively low and constant. We concluded that the level of *glnG* product is low because pgln31 cannot effect the activation of histidase formation (Table 3, experiment 3). We further observed that the low level of *glnG* product provided by pgln31 is not rendered capable of activating histidase formation by eliminating *glnA* and *glnL* products from the cell (Table 3, experiment 4). Thus, the inability of low levels of *glnG* product to activate histidase formation is not a consequence of any regulatory activity of the *glnL* product.

We showed that the ability of the *glnG* prod-

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TABLE 1. Bacteria, phage, and plasmids used

Bacterium/phage/plasmid	Genotype	Source or reference
E. coli		
YMC9	Δ*lacU169 endA thi hsdR supE44*	(1)
YMC10	Δ*lacU169 endA thi hsdR supE44 hutC*$_{Klebs}$	(1)
YMC11	Δ*lacU169 endA thi hsdR supE44 hutC*$_{Klebs}$ Δ(*glnA-glnG*)*2000*	(1)
YMC12	Δ*lacU169 endA thi hsdR supE44 hutC*$_{Klebs}$ *glnG10*::Tn*5*	(1)
YMC21	Δ*lacU169 endA thi hsdR supE44* Δ(*glnA-glnG*)*2000*	(2)
YMC23	Δ*lacU169 endA thi hsdR supE44 hutC*$_{Klebs}$ Δ(*glyA-glnG*)*2000 glnF208*::Tn*10*	YMC11 × ET6059[a]
YMC27	Δ*lacU169 endA thi hsdR supE44* Δ(*glnA-glnG*)*2000 glnF208*::Tn*10*	YMC21 × ET6059[a]
ET6059	*glnF208*::Tn*10*	(8)
Bacteriophage		
λ *gln*101	*glnAp* oriented to transcribe *lacZ*	(1)
λ *gln*103	*glnA*$^+$*L*$^+$*G*$^+$ region carried on phage λ	(2)
λ *gln*104	*glnA*$^+$ Δ*glnL2001 glnG*$^+$ carried on phage λ	(2)
Plasmids		
pgln31	*glnG* cloned in pBR322[b]	This work
pgln53	DNA fragment carrying *glnAp* positioned adjacent to *glnG*	(2)

[a] By P1 transduction.

[b] DNA fragment from a *Sal*I site several hundred base pairs into *glnL* to a *Hin*dII site beyond the end of *glnG* was cloned between the *Sal*I and *Eco*RI sites of pBR322 after conversion of the *Hin*dII site mentioned to an *Eco*RI site by linkers

uct provided in low concentration to regulate the expression of *glnA* does not require the product of the *glnL* gene. This demonstration made use of phage λ *gln*101, which carries a *lacZ* gene transcribed from a *glnA* promoter, but no other *gln* genes (1). Strain YMC21, whose *glnALG* region is deleted, was lysogenized with λ *gln*101 and was found to produce β-galactosidase at a low level; introduction of pgln31 greatly stimulated the production of β-galactosidase (Table 4, experiments 1 and 2). This stimulation depended on a functional *glnF* gene (Table 4, experiment 3). Although in *glnF* mutants the expression of the *glnA* promoter is usually more severely

TABLE 2. Effect of deletion of *glnL* on glutamine synthetase synthesis

Expt	Strain	Glutamine synthetase sp act on:[a]			
		Relevant *gln* genotype	N-limiting medium	N-excess medium	N-excess, C-limiting medium
1[b]	YMC21(λ *gln*103)	*gln*$^+$	950	115	65
2[b]	YMC21(λ *gln*104)	Δ*glnL2001*	1,000	300	55
3	YMC27(λ *gln*103)	*glnF208*::Tn*10*	20	20	ND[c]
4	YMC27(λ *gln*104)	*glnF208*::Tn*10* Δ*glnL2001*	20	20	ND

[a] Enzyme specific activities are given as nanomoles of product formed per minute per milligram of protein determined as previously described (6). Cultures were grown in minimal medium containing 0.4% glucose and 0.2% glutamine (N-limiting medium) or 0.4% glucose, 0.2% glutamine, and 0.2% ammonium sulfate (N-excess medium) or in LB medium containing 0.2% glutamine (N-excess, C-limiting medium) as described previously (7).

[b] These experiments are quoted from reference 2.

[c] ND, Not determined.

TABLE 3. Effects of *glnG* and *glnF* gene products on glutamine synthetase and histidase syntheses

Expt	Strain[a]	Relevant *gln* genotype of host	Growth conditions[a]	Glutamine synthetase sp act[b]	Histidase sp act[b]
1	YMC10	*gln*$^+$	N limiting	1,080	380
			N excess	185	130
2	YMC12	*glnG10*::Tn*5*	N limiting	55	115
			N excess	80	115
3	YMC12(pgln31)	*glnG10*::Tn*5*	N limiting	1,290	150
			N excess	90	115
4	YMC11(pgln31)	Δ(*glnA-glnG*)*2000*	N limiting	NA[c]	60
			N excess	NA	75
5	YMC10(pgln53)	*gln*$^+$	N limiting	320	370
			N excess	45	210
6	YMC11(pgln53)	Δ(*glnA-glnG*)*2000*	N limiting	NA	250
			N excess	NA	255
7	YMC23(pgln53)	Δ(*glnA-glnG*)*2000* *glnF208*::Tn*10*	N limiting	NA	65
			N excess	NA	75

[a] The cultures were grown in minimal medium containing 0.4% glucose and 0.2% glutamine (N limiting) or 0.4% glucose, 0.2% glutamine, and 0.2% ammonium sulfate (N excess) as previously described (7).
[b] Nanomoles of product formed per minute per milligram of protein, as previously described (6).
[c] NA, Not applicable.

reduced than in *glnG* mutants (4), the effect in our experiments was only modest (Table 4, compare experiments 1 and 3). We have not established a reason for this slight discrepancy.

We were also able to show that the activation of histidase synthesis does not require the products of the *glnA* and *glnL* genes when the level of *glnG* product is sufficiently high. In this case, we used plasmid pgln53, in which *glnG* is expressed from an adjacently positioned *glnA* promoter (2). Strains carrying pgln53, whether they carry the *glnALG* region [YMC10(pgln53)] or are deleted for it [YMC11(pgln53)], produced histidase at a high level, irrespective of the presence or absence of ammonia; this activation of histidase synthesis required a functional *glnF* gene (Table 3, experiments 5, 6, and 7). It would appear that in the presence of *glnF* product, expression of genes such as that for histidase responds strictly to the level of *glnG* product and not to other regulators or indicators of ammonia availability. In wild-type cells, in which *glnG* expression is coregulated with *glnA* (6), *glnG* product levels are presumably tightly correlated with ammonia availability.

Finally, it is of interest that in the strain carrying pgln53, the synthesis of glutamine synthetase responded in the normal manner to regulation by ammonia, but that the levels of the enzyme were considerably lower than in the corresponding wild-type strain (Table 2, experiments 1 and 5). It has previously been shown that *glnA* expression can be higher in cells in which the product of *glnG* can only be provided at a low level by transcription initiated at the *glnL* promoter than in cells in which the product can be provided by transcription initiated at the *glnA* promoter (7, 8). It is therefore possible that the *glnG* product can set the limits of the expression of the *glnA* gene without reference to the nitrogen source of the medium.

In summary, we find that the absence of *glnL* product does not eliminate the ability of *glnF* and *glnG* products to regulate expression of *glnA* and other nitrogen-regulated genes, such as histidase, in response to varying ammonia availability. Since the regulatory effects of *glnB* and *glnD* mutations are not manifested in *glnL* deletion strains (2, 5), we believe that *glnF* and *glnG* are the only known genes indispensable for regulation of *glnA* and other nitrogen-regulated genes.

TABLE 4. Effects of *glnG* and *glnF* gene products on *glnA* expression

Expt	Strain	Relevant *gln* phenotype of host	β-Galactosidase sp act[a]
1	YMC21(λ *gln*101)	Δ(*glnA-glnG*)*2000*	300
2	YMC21(λ *gln*101)(pgln31)	Δ(*glnA-glnG*)*2000*	3,950
3	YMC27(λ *gln*101)(pgln31)	Δ(*glnA-glnG*)*2000* *glnF208*::Tn*10*	210

[a] Nanomoles of product formed per minute per milligram of protein, determined as previously described (6).

Regulation in response to nitrogen source thus appears to consist of a central process mediated by the products of *glnF* and *glnG* and subjected to modulation by the products of other genes. The role of the products of *glnD* (uridylyltransferase) and of *glnB* (P_{II}), both of which are also components of the system responsible for the regulation of glutamine synthetase activity by covalent modification (9), appears to be the accurate assessment of the availability of ammonia. Our data suggest that the role of the *glnL* product is the transmission of *glnB-glnD*-derived information to the *glnF-glnG* regulatory system. This redundant assessment of ammonia availability presumably permits extremely fine control of expression of the regulated genes.

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